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Glutamate antagonists prevent morphine withdrawal in mice and guinea pigs

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The effects of excitatory amino acid antagonists on increased cortical acetylcholine release and behavioural hyperactivity induced by naloxone in morphine tolerant guinea pigs and mice were studied. The results show that the *N*-methyl-D-aspartic acid (NMDA) antagonist MK-801 (0.1–1 mg/kg, i.p.) injected 30 min before naloxone (3 mg/kg, s.c.) dose-dependently prevented the neurochemical and behavioural signs of morphine withdrawal in guinea pigs and mice. The non-selective antagonist glutamic acid diethylester only at 100 mg/kg i.p. reduced the naloxone-induced increase of cortical acetylcholine release without affecting the behavioural changes. These findings indicate that the activation of excitatory amino acid receptors, mainly the NMDA receptors, plays a relevant role in the expression of opiate abstinence.

Naloxone(Nx)-precipitated morphine withdrawal in tolerant animals is characterized by behavioural, EEG and neurochemical changes [8, 11] such as hyperactivity and jumping in mice and rats [6, 10, 12], reversal of diazepam-induced EEG synchronization (unpublished observations) and increased cortical acetylcholine (ACh) release in guinea pigs [1, 3, 4]. Recent electrophysiological studies indicate that rat locus coeruleus (LC) cells receive an excitatory input by amino acidergic neurons (EAA) located in the paragigantocellularis nucleus (PN). This excitatory control is reported to be enhanced during morphine withdrawal [9, 13].

To gain further insight into the role of the EAA system in morphine withdrawal, the effects of 3 EAA antagonists on Nx-induced behavioural changes in tolerant mice and on cortical ACh release in tolerant guinea pigs was investigated.

Opiate dependence in mice was induced by subcutaneous (s.c.) implantation of one morphine pellet (75 mg). Two days later, the animals were tolerant, since Nx injection (ineffective in naive mice) induced a dose-dependent jumping and hyperactivity [14]. To quantify the scores, each mouse was placed separately in a Perspex box. Scores were taken 10 min before and until 15 min after injection of Nx (3 mg/kg, s.c.).

Opiate dependence in guinea pig was induced by s.c. implantation of morphine base pellets (75 mg), one pellet on the 1st day and two pellets on the 4th day of treatment. On the 5th day an epidural cup was implanted on the left or right parietal bone [2] to measure ACh outflow from the underlying parietal cortex. On the 7th day the release experiment was carried out (for details see ref. 4). Pre-treatment with the various EAA antagonists was performed 30 min before Nx in mice and guinea pigs.

As shown in Fig. 1, various doses of the non-selective EAA antagonist glutamic acid diethylester, injected i.p. 30 min prior to Nx 3 mg/kg s.c. did not affect the frequency of jumping in mice. However, the more selective NMDA antagonist, MK-801 (at 0.3 and 1 mg/kg but not 0.1 mg/kg, i.p.) completely prevented it. Since MK-801 at the highest dose induced ataxia, this side effect might be suspected to overshadow the signs of the withdrawal. However, any role of ataxia was ruled out because diazepam at 2 mg/kg i.p. caused ataxia but failed to antagonize the signs of abstinence. Pyroglutamic acid, a non-NMDA antagonist [5], was able to counteract Nx-induced jumping in the mice only at 1 g/kg dose i.p.

As previously reported [1] naloxone (3 mg/kg, i.p.), produced a 3-fold-increase in cortical ACh outflow in morphine-tolerant guinea pigs. This facilitatory effect, as well as the signs of behavioural abstinence (exploring, wet dog shakes, jumping) was partly prevented by glutamic acid diethylester (100 mg/kg, i.p.) and was completely counteracted by MK-801 (1 mg/kg, i.p.). The antagonism of MK-801 was still evident at the dose of 0.3

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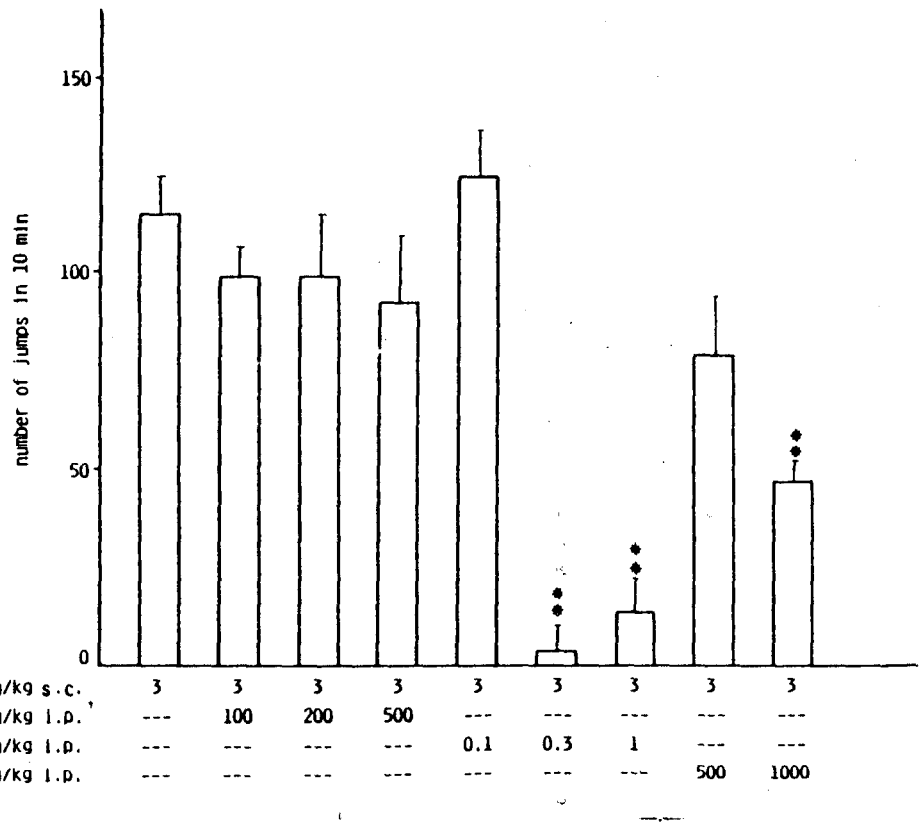

Fig. 1. Effect of pretreatment with glutamic acid diethylester (GDEE), MK-801, pyroglutamic acid (Pyro) on the number of jumps induced by naloxone (Nx) (3 mg/kg, s.c.) in tolerant mice. Significantly different from Nx 3 mg/kg alone. $^{**}P<0.01$ according to the ANOVA analysis followed by Newman-Keuls multiple range test. Each column is the mean ± S.E.M. of 15–20 animals.

mg/kg i.p. (Fig. 2) but not at 0.1 mg/kg i.p. (data not shown). Conversely, pyroglutamic acid (1 g/kg i.p.), did not prevent the increase in cortical ACh outflow in guinea pigs.

The present results show that cortical ACh release in the guinea-pig and jumping in the mouse (assumed as indices of Nx-precipitated withdrawal) are fully prevented by pretreatment with MK-801 and partially antagonized by glutamic acid diethylester. Thus the activation of EAA neurons plays a relevant role in the expression of opiate abstinence. This effect is probably mediated mainly through NMDA receptors. In fact, the most active compound was MK-801, a non-competitive antagonist of NMDA receptor, while the much less selective glutamic acid diethylester antagonized only the neurochemical effects. Finally, pyroglutamic acid, which prevents kainate- (but not NMDA) induced convulsions in the mice [5] was unable to modify the increase in cortical ACh release in the guinea pig and only reduced the frequency of jumping in mice. These data are consistent with the electrophysiological findings of Rasmussen et al. [13] which indicate that during morphine-withdrawal the activation of locus coeruleus is partly mediated by EAA projections from the paragigantocellularis nucleus, through kinurenate-sensitive receptors. In turn, the increased firing rate of central noradrenergic cells, contributes to expression of the opiate abstinence syndrome. However, other neuronal systems not influenced by LC [4, 7] can participate in the neurochemical and behavioural end-effects. Further studies on morphine-tolerant animals, performed by measuring different neurotransmitters released from various brain areas, might be relevant to establish the actual role played by the NMDA receptors.

In conclusion the present study shows that EAA release during withdrawal appears to represent an essential step in the expression of opioid abstinence.

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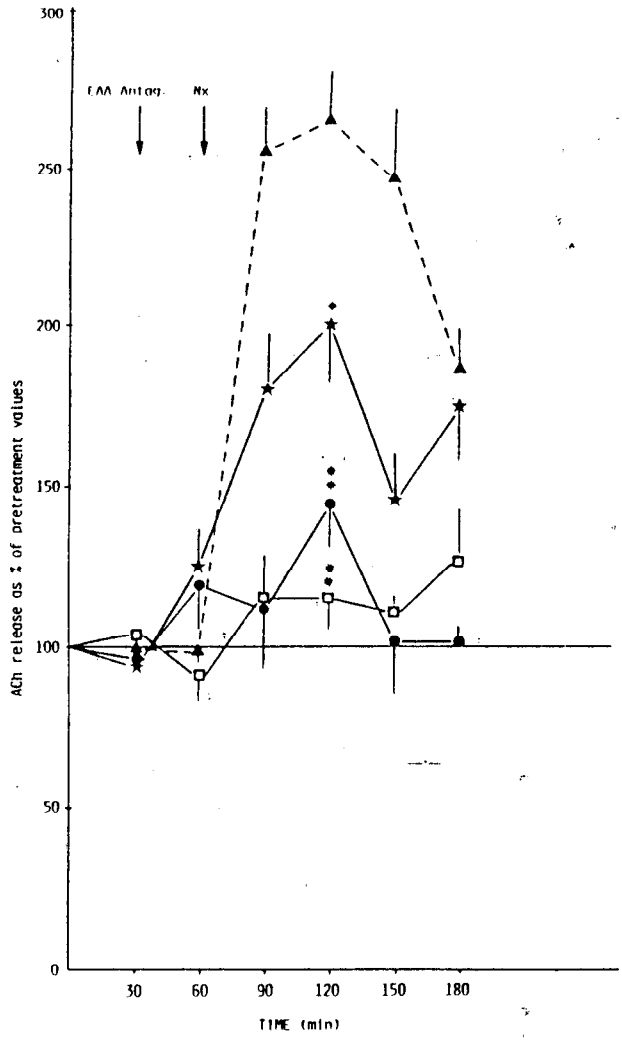

Fig. 2. Percent changes in cortical ACh release in morphine-tolerant guinea pigs induced by naloxone (Nx, ▲, 3 mg/kg i.p.) alone and 30 min after MK-801 (0.3, ★; 1 mg/kg, i.p., □) or glutamic acid diethylester (100 mg/kg, i.p., ●). The drugs were injected as indicated by the arrows. Cortical ACh outflow was expressed as percent of the mean of basal release (92 ± 2 pmol/cm^2/30 min). Each value is the mean $\pm$S.E.M. of 5–6 animals. $^*P < 0.05$, $^{**}P < 0.01$. The statistical analysis was carried out by a one-way analysis of variance followed by the Newman–Keuls multiple range test.

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